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ACID–BASE AND ION BALANCE, METABOLISM, AND THEIR INTERACTIONS, AFTER EXHAUSTIVE EXERCISE IN FISH

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Summary

In fish, exhaustive exercise stress differs from steady-state aerobic exercise in causing (1) a depletion of glycogen, creatine phosphate (CP) and ATP reserves and an accumulation of lactate and metabolic acid (H_m^+) in white muscle; (2) blood respiratory and metabolic acidoses (P_{CO_2} and H_m^+ elevations, respectively); (3) marked ionic and fluid volume disturbances; and (4) a surge in plasma catecholamines. During recovery, the smaller fast component (20%) of excess post-exercise oxygen consumption (EPOC) is explained by CP and ATP resynthesis and aerobic demands, but the larger slow component (80%) is considerably greater than the cost of lactate clearance and glycogen resynthesis. Ionic and H_2O shifts may contribute significantly to EPOC; net fluxes are greatest between extracellular (ECF) and intracellular fluid (ICF) compartments, with smaller disturbances at the kidney (increased filtration, reabsorption and excretion) and gills (passive ion losses and H_2O uptake). Modulation of branchial Na^+ and Cl^- exchange is important in the temporary storage of H_m^+ in the environment during recovery. Movements of lactate and H_m^+ from ICF to ECF are dissociated processes; the major portions of both are retained in the white muscle and are probably cleared by oxidation and/or glycogen resynthesis *in situ*. Elevated catecholamine levels are implicated in many of these responses and serve to protect metabolic processes against acid–base disturbances, but do not appear to contribute to EPOC directly. Catecholamines also cause an elevation in blood P_{CO_2} by a mechanism linked to the β-adrenergic activation of red blood cell Na^+/H^+ exchange that protects O_2 transport. The compound blood acidosis stimulates ventilation to meet the demands of EPOC.

Introduction

An experimental exercise protocol variously described as 'intensive', 'strenuous' or 'exhaustive activity', or more accurately as 'exhaustive exercise stress', has been intensively exploited over the past 15 years. In its least invasive form, the procedure involves swimming the fish at progressively higher speeds in a swim tunnel until the maximum sustainable velocity (U_{crit}) is reached, followed by burst

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swimming at higher velocity to ensure exhaustion (Butler *et al.* 1986; Primmet *et al.* 1986; Mommsen and Hochachka, 1988). More commonly, the fish is manually chased to exhaustion in a small tank or trough (Turner *et al.* 1983*a*; Milligan and Wood, 1986*a*; McDonald *et al.* 1989*a*), a technique first used extensively 30–40 years ago in the pioneering work of E. C. Black (Black, 1958; Black *et al.* 1962). In another variation, electric shocks, either alone or in combination with chasing, are employed to ensure complete exhaustion (Holeton *et al.* 1983; Wieser *et al.* 1985; Pearson *et al.* 1990). The endpoint is clearly manifested as an inability to perform further burst exercise (slow swimming *via* aerobic red muscle generally persists), and accompanying physiological changes are remarkably uniform within a particular batch of fish.

The validity of this approach in duplicating 'natural' exhaustive exercise has been much debated, especially by referees, but it is equally debatable whether truly natural exercise is ever exhaustive in fish. However, through man's intervention in the natural environment, the phenomenon is now a common occurrence. Examples include angling (especially catch and release sportfishing; Bouck and Ball, 1966; Wydoski *et al.* 1976; Beggs *et al.* 1980; Schwalme and Mackay, 1985*b*; Wells *et al.* 1986), commercial fishing 'throwback' (Parker and Black, 1959; Neilsen *et al.* 1989), netting and electrofishing for biological surveys (Schreck *et al.* 1976; Mesa and Schreck, 1989), and transportation, confinement and handling stresses resulting from aquacultural and fish-stocking practices (Fraser and Beamish, 1969; Barton *et al.* 1986; Woodward and Strange, 1987). The physiological disturbances resulting from exhaustive exercise stress may be so severe as to cause the death of the animal several hours later (Black, 1958; Wood *et al.* 1983).

The applied relevance notwithstanding, studies on exhaustive exercise stress in fish have yielded a wealth of basic physiological knowledge. The process of exhaustion itself is rapid, the physiological condition changing from rest or steady-state aerobic exercise to glycolytic exhaustion within a few minutes. As yet, there is little information on the sequence of events occurring during this brief period of dynamic change. The original study of Black *et al.* (1962) is still the most detailed on time course, while Parkhouse *et al.* (1987, 1988) have provided some modern biochemical information. However, most of our new knowledge is of the consequences prevailing once exhaustion has occurred, and the changes that take place during post-exercise recovery. The slow time course (4–24 h) of this recovery has greatly facilitated dissection of the mechanisms involved. The present review presents an overview of these responses following exhaustive exercise stress, and focuses on some areas of particular interest or controversy. Randall and Brauner (1991, this symposium) review aerobic exercise in fish.

Exhaustive *versus* aerobic exercise

Several physiological responses distinguish exhaustive exercise from steady

late aerobic exercise (Wood and Perry, 1985). Selected aspects are illustrated in Fig. 1.

There is a significant depletion of glycogen reserves and an associated large build-up of lactate in white muscle caused by the anaerobic glycolysis used for burst performance (e.g. Black *et al.* 1962; Milligan and Wood, 1986*b*; Dobson and Hochachka, 1987). Blood lactate levels may rise substantially (Fig. 1B), though never to the levels seen in white muscle (see Fig. 4). There is also a substantial degradation of muscle ATP and creatine phosphate stores, to levels far lower than those seen at exhaustion in many higher vertebrates (Driedzic and Hochachka, 1976; Dobson *et al.* 1987; Mommsen and Hochachka, 1988). While some glycolytic generation of lactate occurs during steady-state swimming, production and oxidation rates appear to reach equilibrium so that elevations of blood and muscle lactate levels are small (Johnston and Goldspink, 1973; Driedzic and Kiceniuk, 1976; Wokoma and Johnston, 1981; Duthie, 1982; Boutilier *et al.* 1984; Butler *et al.* 1986). Muscle ATP and creatine phosphate levels are not depressed in aerobically swum fish (Parkhouse *et al.* 1987; Dobson and Hochachka, 1987).

A marked depression of blood pH (Fig. 1A) occurs in all fish after exhaustive exercise stress (Wood *et al.* 1977; Jensen *et al.* 1983; Turner *et al.* 1983*a*,*b*; Holeton and Heisler, 1983; Schwalme and Mackay, 1985*a*; Walsh, 1989). This results from a combination of metabolic (H_m^+ elevation; Fig. 1B) and respiratory acidoses (P_{CO_2} elevation; Fig. 1C). While the cause of P_{CO_2} elevation has been controversial (see below), metabolic acidosis has generally been attributed to protons generated in association with lactate production and ATP breakdown in white muscle (Hochachka and Mommsen, 1983; Wood and Perry, 1985). It is now clear that proton extrusion from red blood cells may also play an important role in some species (see below). In contrast, during sustained aerobic exercise, blood acid–base status exhibits only minimal changes from control levels (Kiceniuk and Jones, 1977; Boutilier *et al.* 1984; Butler *et al.* 1986; Thomas *et al.* 1987).

Exhaustive exercise stress causes a profound disturbance of ionic, osmotic and fluid volume homeostasis. A large net fluid shift from extracellular to intracellular compartments results from the osmotic attraction offered by greatly increased intracellular lactate levels (Milligan and Wood, 1986*a*,*b*, 1987*b*,*c*). Haemoconcentration occurs, and concentrations of all major plasma electrolytes generally increase (Fig. 1D) because of this decrease in plasma water (Turner *et al.* 1983*a*,*b*; Holeton *et al.* 1983; Thomas *et al.* 1987; van Dijk and Wood, 1988). Plasma electrolyte levels rise, despite net losses through the gills and kidney in freshwater fish (Wood and Randall 1973*a*; Wood, 1988). In contrast, the limited observations available on fish undergoing steady-state exercise indicate minimal changes in levels of plasma electrolytes (Rao, 1969; Farmer and Beamish, 1969; Parkhouse *et al.* 1987; Thomas *et al.* 1987) and branchial ion flux rates (Wood and Randall, 1973*b*). However, it is interesting that both exercise regimes cause increased branchial water entry and compensating renal excretion (Wood and Randall, 1973*c*; Hofmann and Butler, 1979; Wood, 1988). Increased water flux is presumably an unavoidable correlate of increased O_2 diffusing capacity at the gills.

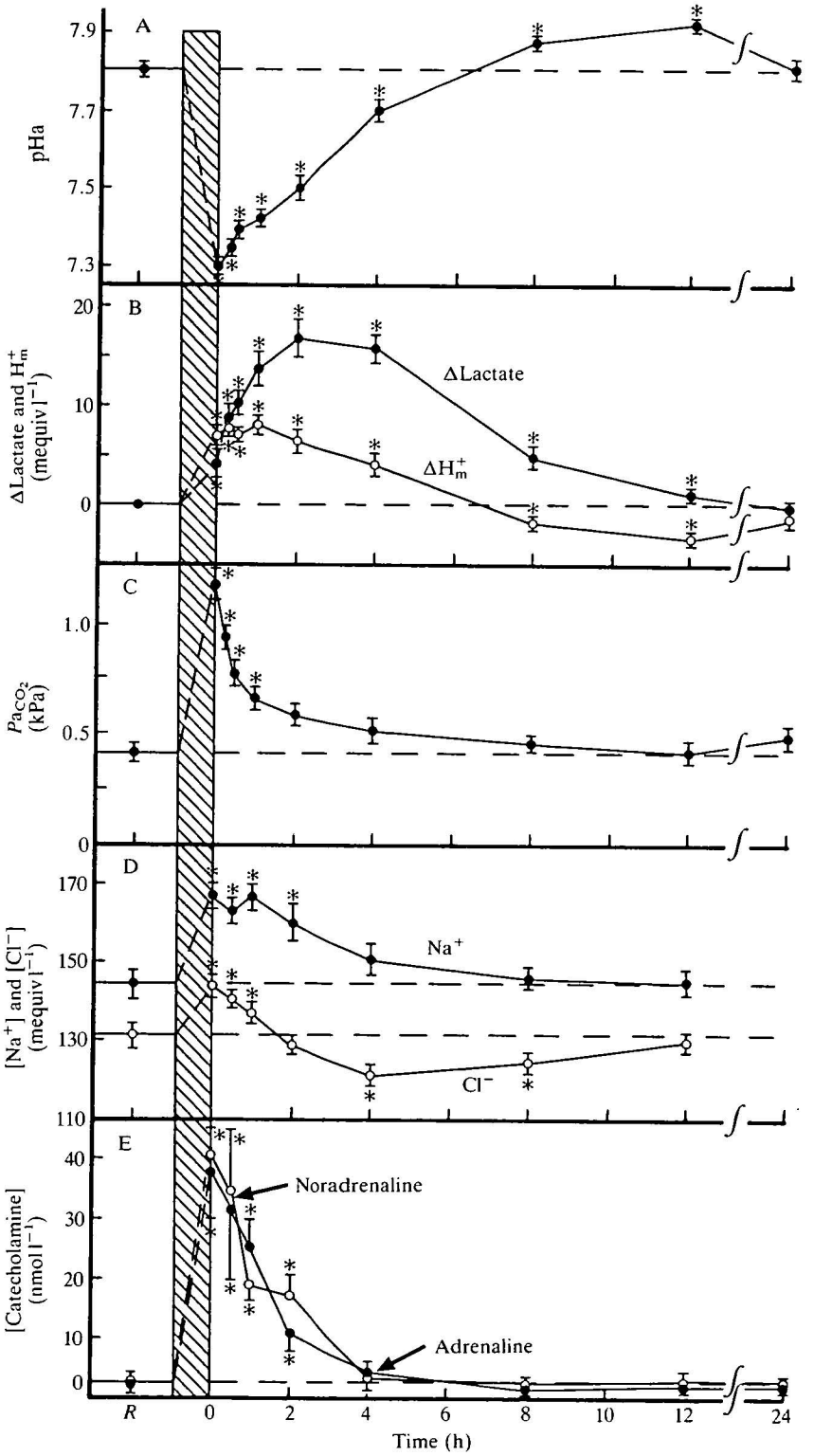
A
pHa
7.9
7.7
7.5
7.3
B
ΔLactate and H_m^+ (mequiv l^{-1})
20
10
0
ΔLactate
ΔH_m^+
C
P_{aCO_2} (kPa)
1.0
0.5
0
D
[Na^+] and [Cl^-] (mequiv l^{-1})
170
150
130
110
Na^+
Cl^-
E
[Catecholamine] (nmol l^{-1})
40
30
20
10
0
Noradrenaline
Adrenaline
R
0
2
4
6
8
10
12
24
Time (h)

Fig. 1

Fig. 1. Some physiological responses diagnostic of exhaustive exercise stress (6 min chasing, at bar) in adult freshwater rainbow trout and changes in these variables over the following 24 h of recovery at 15 °C. (A) Arterial pH (pHa); (B) whole-blood lactate (Δlactate) and metabolic acid (ΔH_m^+) loads; (C) arterial CO_2 tension (Pa_{CO_2}); (D) plasma sodium and chloride levels; and (E) plasma noradrenaline and adrenaline levels. A, B and C are from Milligan and Wood (1986*a*), D is from Turner *et al.* (1983*a*) and E is from Milligan and Wood (1987*a*). Means±1 S.E.M., *N* numbers as in original papers. *R* and dashed line indicate pre-exercise resting level; * indicates a mean value significantly different ($P<0.05$) from rest.

An important difference between exhaustive and sustained exercise, and one that may play a key role in explaining other differences, is the extent of catecholamine mobilization. It is now clear that fish performing sustained aerobic exercise exhibit little or no increase in circulating catecholamine levels (Ristori and Laurent, 1985; Butler *et al.* 1986; Primmet *et al.* 1986; Axelsson and Nilsson, 1986; Hughes *et al.* 1988). However, once U_{crit} is surpassed and burst performance starts, there is a rapid release of adrenaline and noradrenaline into the bloodstream (Primmett *et al.* 1986; Butler *et al.* 1986, 1989). This mobilization seems to be particularly pronounced when the stress component is high, as in chasing to exhaustion (Fig. 1E; Milligan and Wood, 1987*a*; Tang and Boutilier, 1988; van Dijk and Wood, 1988; Milligan *et al.* 1989; Walsh, 1989; Wood *et al.* 1990*b*). Levels of both catecholamines (around 10^{-9} mol l^{-1} at rest) may increase by 1–2 orders of magnitude; Milligan and Wood (1987*a*), Tang and Boutilier (1988) and Perry *et al.* (1989) provide useful tabulations of the recent literature.

The proximate stimulus for catecholamine release remains unclear. Some workers have related the phenomenon to acidosis (Boutilier *et al.* 1986; Tang and Boutilier, 1988), others to a decline in blood O_2 content (Perry *et al.* 1989; Fievet *et al.* 1990). It is quite possible that both stimuli are involved. The discharge of catecholamines from chromaffin tissue appears to be directly responsive to the chemical characteristics of the perfusing venous blood (Perry *et al.* 1991*a*). Venous blood becomes both acidic and severely hypoxaemic once U_{crit} is exceeded (Kiceniuk and Jones, 1977; Butler *et al.* 1989). A psychological component ('panic'), acting through the sympathetic innervation of the chromaffin cells, is also quite possibly involved. Section of the sympathetic spinal nerves to the head kidney reduces catecholamine mobilization during stress (Nilsson *et al.* 1976; Butler *et al.* 1989; Perry *et al.* 1991*a*).

The metabolic cost of post-exercise recovery

From the above discussion, it is clear that the physiological state of the fish after exhaustion is very different from that prior to exercise. The task of recovery is to restore the original condition with as little additional metabolic cost as possible. This cost can be quantified as the 'excess post-exercise oxygen consumption' or EPOC. The measurements of Brett (1964) on salmon are still the most detailed; these indicate that EPOC lasts 4–6 h after exhaustion and would be large enough

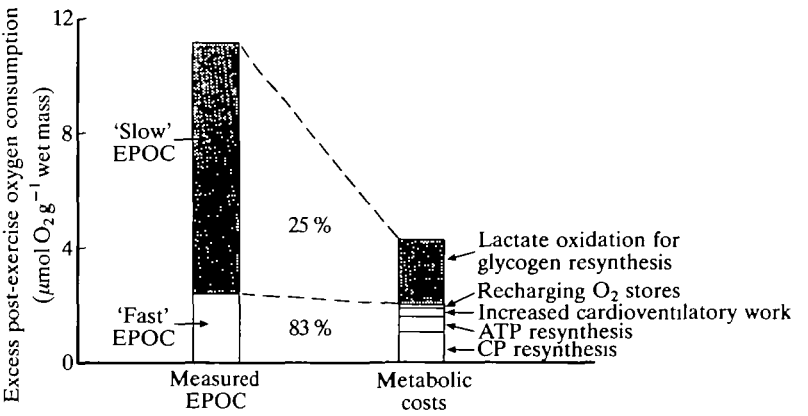

Fig. 2. The measured 'fast' and 'slow' components of excess post-exercise oxygen consumption (EPOC) in juvenile rainbow trout after exhaustive exercise stress (5 min chasing) at 15°C, and their possible origins. Most (83 %) of the fast component is accounted for by creatine phosphate (CP) and ATP resynthesis, increased cardioventilatory work and the recharging of body O_2 stores. However, only 25 % of the slow component is explained by the metabolism of lactate back to glycogen. Based on data from Scarabello *et al.* (1991*a*).

to support about 1 h of aerobic swimming at U_{crit}. The traditional view of mammalian physiology, formulated by A. V. Hill, R. Margaria and their associates in the 1920s (Hill and Lupton, 1923; Margaria *et al.* 1933), and still found in many textbooks today, has been that most of the EPOC is accounted for by the metabolism of lactate back to glycogen. However, modern re-assessments have concluded that this 'oxygen debt hypothesis' is overly simplistic. A host of other metabolic disturbances besides lactate clearance are now thought to contribute to EPOC in mammals; the most important of these is the post-exercise elevation in body temperature (see Gaesser and Brooks, 1984, for a review). In poikilothermic fish, temperature elevation is clearly not a factor; it is surprising, therefore, that no detailed assessments of the oxygen debt hypothesis in fish have been published.

Recently, Scarabello *et al.* (1991*a*) have performed such an assessment by making frequent measurements of the rate of oxygen consumption ($\dot{M}_{O_2}$) and metabolite levels in the whole body of juvenile trout during recovery from exhaustive exercise stress. As in mammals (Margaria *et al.* 1933), it was possible to resolve total EPOC into two components by exponential analysis. A 'fast' component (half-time=0.23 h) constituted about one-fifth of the total, and a 'slow' component (half-time=2.1 h) accounted for the other four-fifths (Fig. 2). Most (83 %) of the fast component could be accounted for by the same factors as in mammals – measured restoration of creatine phosphate and ATP stores and reasonable estimates of the costs of increased cardioventilatory work and the recharging of body O_2 stores. However, only about 25 % of the larger slow component could be explained by measured rates of lactate clearance an

glycogen resynthesis and a metabolic scenario based on the classical 'oxygen debt hypothesis', i.e. glycogen resynthesis as the primary fate of lactate. A scenario assuming oxidation as the primary fate of lactate produced a similarly unreasonable result. EPOC was far larger than could be explained by lactate metabolism and glycogen resynthesis.

To confirm this conclusion, tests were performed to see whether EPOC could be experimentally dissociated from lactate clearance. In fish depleted of glycogen by starvation prior to exercise, both lactate clearance and glycogen resynthesis were reduced by 30%, but EPOC was unaltered (Scarabello *et al.* 1991*b*). Conversely, in fish subjected to a second exercise bout 6 h after the first, EPOC was reduced by 40%, but lactate clearance and glycogen resynthesis were unchanged (M. Scarabello, G. F. Heigenhauser, and C. M. Wood, unpublished results). Clearly, EPOC and post-exercise lactate metabolism are not quantitatively related. Several other studies have also discounted a tight relationship between EPOC and lactate metabolism in fish, for different reasons (Wieser *et al.* 1985; Milligan and McDonald, 1988). Even though temperature elevation cannot be involved, other factors must be sought to explain the major portion of EPOC in fish.

Ion, acid-base and fluid volume disturbances

Important contributors to EPOC may be the costs of altered membrane transport processes associated with re-establishing ion, acid–base and fluid volume homeostasis. The major focus of research has been on the gills, the closest point of contact of the extracellular fluid with an osmotically hostile evironment. However, as illustrated by Fig. 3, the kidney and the ECF/ICF interface actually exhibit much larger net fluxes, a point that has been generally overlooked.

At the gills, net losses of major electrolytes and gains of water in freshwater fish (and the opposite in seawater fish) may continue for some time during recovery from exhaustive exercise (Fig. 3); these deficits must eventually be corrected by the increased expenditure of metabolic energy. Elevated water and O_2 fluxes are probably linked by simple permeability effects associated with increased branchial surface area, reduced diffusion distance and catecholamine mobilization (see Wood and Perry, 1985). The same factors will influence ion fluxes, but the situation is complicated by the dynamic modulation of branchial ion exchanges for the purpose of acid–base correction. Metabolic acid (H^+_m) released from white muscle is transiently shuttled to the external environment across the gills (Fig. 3), and then later retrieved for final metabolic correction (Holeton and Heisler, 1983; Milligan and Wood, 1986*a*, 1987*b*; Milligan *et al.* 1991). At least in freshwater fish, the branchial movements of H^+_m are linked to movements of strong ions (Holeton *et al.* 1983; Wood, 1988; McDonald *et al.* 1989*a*,*b*). During early recovery, active Na^+ influx (Na^+/acid exchange) is stimulated while Cl^- influx (Cl^-/base exchange) is inhibited, thereby achieving net H^+_m excretion. During later recovery, these trends are reversed, thereby achieving net H^+_m uptake. Modulation of branchial ion exchange may be a direct response to internal acid–base status

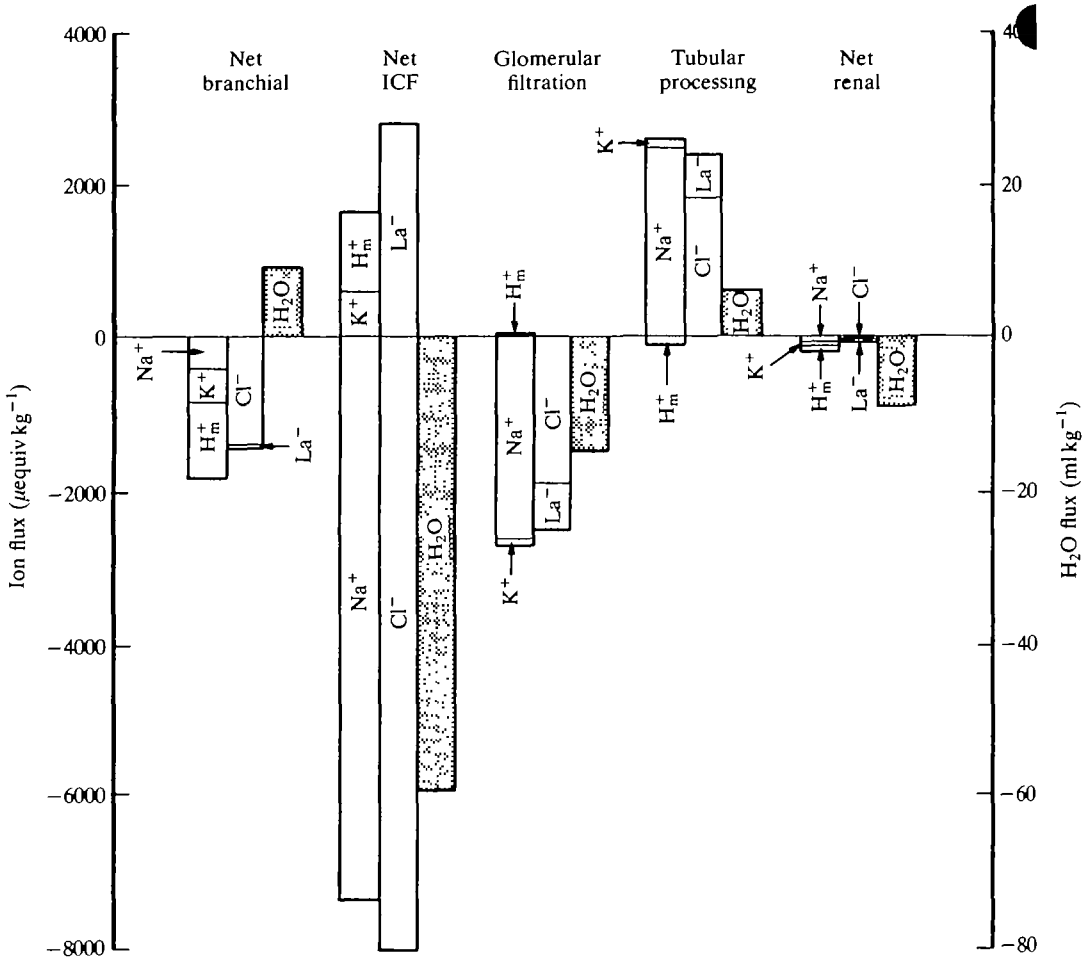

Fig. 3. Cumulative net fluxes of major inorganic ions (Na^+, Cl^-, K^+), metabolic acidic equivalents (H_m^+), lactate (La^-) and H_2O over the first 6 h of recovery from exhaustive exercise stress (6 min chasing) in adult freshwater rainbow trout at 15°C. For each flux site, the first bar represents cation flux, the second bar anion flux and the third bar H_2O flux. The direction of each flux is shown relative to the extracellular fluid (ECF) compartment; losses from the ECF are negative, gains are positive. Composite data from Turner *et al.* (1983*a*), Milligan and Wood (1986*a*) and Wood (1988). ICF, intracellular fluid.

(Milligan *et al.* 1991; Goss and Wood, 1991) and/or catecholamines may play a controlling role (Wood and Perry, 1985; Vermette and Perry, 1987; McDonald *et al.* 1989*a*,*b*). This temporary 'storage' of H_m^+ in the water represents only a small fraction of the whole-body load, but is important in preventing intolerable acidosis in the poorly buffered extracellular compartment. Almost no lactate anion is lost across the gills (Holeton and Heisler, 1983; Milligan and Wood, 1986*a*).

The kidney makes only a very small contribution to acid–base regulation after exercise, and urinary lactate losses are also minimal (Fig. 3; Holeton and Heisle

983; Holeton *et al.* 1983; Wood, 1988). In freshwater fish, urine flow rate increases substantially in response to elevated glomerular filtration rate, thereby compensating for increased water uptake at the gills (Wood and Randall, 1973*c*; Hofmann and Butler, 1979; Wood, 1988). However, renal ion losses are only slightly elevated (Fig. 3). This conservation of ions in the face of increased filtered load is achieved by large elevations in the tubular reabsorption of Na^+, Cl^-, lactate and other electrolytes (Fig. 3). The changes in net tubular fluxes are actually larger than those at the gills during the first 6 h after exercise in trout. Most of the metabolic expenditure of the kidney is probably incurred in active tubular reabsorption, and so would increase by about 60 % during this period.

By far the greatest ion and fluid disturbances occur at the ECF/ICF interface. In freshwater trout, a shift of water out of the extracellular compartment occurs which is about sixfold greater than the elevated water entry at the gills (Fig. 3). This shift is largely entrained by the osmotic effect of the high muscle lactate load (Fig. 4; Milligan and Wood, 1986*a*,*b*). Swelling of red blood cells (RBCs) in response to acidosis and elevated plasma catecholamine levels also occurs (Nikinmaa, 1982; Primmett *et al.* 1986; Milligan and Wood, 1987*a*; Milligan *et al.* 1989; Wood *et al.* 1990*b*), but can account for less than 10 % of the observed response.

There are also large net shifts of Na^+ and Cl^- into the intracellular compartment which are 5- to 20-fold greater than their net losses through the gills (Fig. 3). In contrast, a smaller net flux of K^+ into the extracellular compartment occurs, similar in quantity to the branchial loss of K^+. The site(s) of these fluxes are unknown. However, again it is probable that white muscle plays an important role. Part of the Cl^- influx could be coupled with lactate efflux, and part of the Na^+ influx with H_m^+ efflux. In mammals, exhausted acidotic muscle becomes more permeable and slightly depolarized, gaining Na^+, Cl^- and water and losing K^+ (Sjogaard, 1986; Kowalchuk *et al.* 1988). The post-exercise catecholamine surge may also be involved; catecholamines are known to generally increase cellular permeability to Na^+ and K^+ (Horwitz, 1979). Catecholamines also cause large net entries of Na^+ and Cl^- into fish RBCs, fluxes that are associated with intracellular pH (pHi) regulation (Ferguson and Boutilier, 1989; Nikinmaa *et al.* 1990). At most, this RBC response can account for only 10 % of the observed net ECF/ICF Na^+ and Cl^- shifts, but a similar phenomenon occurring to only a very slight extent in other tissues throughout the body would explain the observed data. The metabolic costs of restoring these massive shifts at the ECF/ICF boundary could well be greater than those incurred at the gills and kidney.

Lactate and metabolic acid movements

H_m^+ is produced in excess of lactate in white muscle after exhaustive exercise stress, probably because of degradation of ATP stores (Milligan and Wood, 1986*b*, 1987*c*). The movements of lactate and H_m^+ out of white muscle into the extracellular compartment appear to be dissociated processes. Active, pelagic fish

(e.g. trout, dogfish, tuna) may release appreciable quantities of lactate such that the lactate load considerably exceeds the metabolic acid load in the blood (Figs 1B, 4A; Black *et al.* 1962; Piiper *et al.* 1972; Turner *et al.* 1983*a*; Holeton and Heisler, 1983; Perry *et al.* 1985; Milligan and Wood, 1986*a*,*b*; Dobson and Hochachka, 1987; Mommsen and Hochachka, 1988). In salmonids and some other species, this discrepancy occurs despite the fact that part of the extracellular ΔH_m^+ results from β-adrenergically stimulated H^+ extrusion from RBCs (e.g. Nikinmaa, 1982; Nikinmaa *et al.* 1984; McDonald *et al.* 1989*a*; Wood *et al.* 1990*b*). More sluggish, benthic species (e.g. flatfish, sea raven) release almost no lactate; blood lactate levels remain extremely low, generally less than 2 mequiv l^{-1}, considerably below ΔH_m^+ (Fig. 4B; Wardle, 1978; Turner *et al.* 1983*b*; Milligan and Farrell, 1986; Milligan and Wood, 1987*a*,*b*,*c*; Milligan *et al.* 1991). These differences probably reflect strategies for recovery that are adaptive for pelagic *versus* benthic lifestyles, as discussed by Wood and Perry (1985). However, the key point is that the overall release is small; in both strategies and at all times after exhaustive exercise, most of the lactate and H_m^+ is retained in the white muscle (Fig. 4) for apparent metabolism *in situ* (see below).

Even at rest, lactate concentrations in white muscle ICF are far greater than in extracellular fluid (Fig. 4). Intracellular $[H^+]$ is also greater than extracellular $[H^+]$: pHi is 0.23 (flounder; Milligan and Wood, 1987*c*) to 0.56 units (trout; Milligan and Wood, 1986*a*) lower than extracellular pH (pHe). Assuming a typical membrane potential of −83 mV for fish muscle, both lactate and H^+ distributions are maintained well out of electrochemical equilibrium (Wright *et al.* 1988). Intracellular [lactate] is too high and intracellular $[H^+]$ too low to occur as a result of a passive distribution; the situation must be supported by the expenditure of metabolic energy. Despite the large measured changes in intracellular and extracellular [lactate] and in pHe and pHi caused by exhaustive exercise, calculations based on these data suggest that these disequilibria either do not change appreciably (flounder) or decline slightly (trout) relative to the resting state. However, the metabolic cost of maintaining them will increase approximately in proportion to the passive rates of H^+ and lactate leakage. The labelled lactate turnover studies of Milligan and McDonald (1988) indicate that these leakage rates increase threefold (flounder) to ninefold (salmon) during the 0 – 2 h period of post-exercise recovery.

As yet, we know little about the mechanisms of, or the controls for, transmembrane H_m^+ and lactate flux. Blood flow to white muscle increases substantially after exhaustive exercise (Wardle, 1978; Neumann *et al.* 1983), so perfusion limitations are probably not involved in the retention phenomena. However, in light of the preceding discussion it seems likely that lactate retention involves an active *inward* transport from ECF to muscle ICF. This idea is supported by the experiments of Batty and Wardle (1979) on plaice after exercise, using labelled lactate. An apparent net uptake of lactate into white muscle occurred against the concentration and electrical gradients. Similarly Turner and Wood (1983) demonstrated that treatment with SITS, an anion exchange blocker

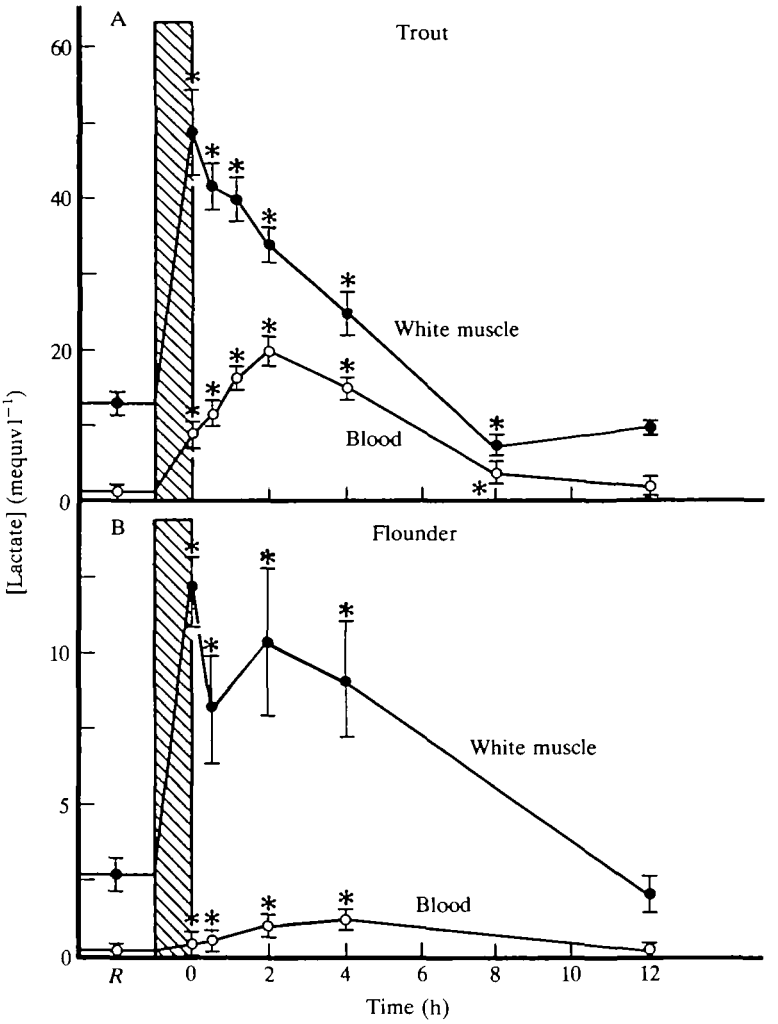

Fig. 4. Changes in white muscle intracellular lactate and whole-blood lactate concentrations after exhaustive exercise stress (at bar: 6 min chasing for trout at 15°C, 10 min for flounder at 11°C) in (A) adult freshwater rainbow trout and (B) adult seawater starry flounder. A is from Milligan and Wood (1986*b*); B is from Milligan and Wood (1987*c*). Means±1 S.E.M., *N* numbers as in original papers. *R* indicates pre-exercise resting level; * indicates a mean value significantly different ($P<0.05$) from rest.

increased net lactate release from a perfused post-exercise trunk preparation of the trout. In plaice, Wardle (1978) provided pharmacological evidence that catecholamine mobilization, acting *via* β-adrenoreceptors, played an important role in preventing lactate release from white muscle. However, repeated attempts to confirm this phenomenon, in both flatfish (Wood and Milligan, 1987) and trout (van Dijk and Wood, 1988; McDonald *et al.* 1989*a*; Tang *et al.* 1989), have proved unsuccessful. β-Adrenergic stimulation does appear to lower blood ΔH_m^+ after

exercise, despite a stimulation of proton extrusion from RBCs, but this can be explained by a stimulation of H_m^+ efflux across the gills, rather by than an effect on H_m^+ release from white muscle.

The model calculations of Holeton and Heisler (1983) for dogfish suggest that the net movement of H_m^+ from muscle ICF to ECF is subject to 'equilibrium limitation' such that H_m^+ efflux falls as pHe falls. This idea is supported by the observation that net H_m^+ release from the perfused trout trunk is inhibited by reduced pHe, while lactate release is unaffected (Turner and Wood, 1983). It is debatable whether the process is passive, because in both cases the electrochemical gradient still favours the net *inward* movement of H^+ from ECF to ICF. However, an analysis based solely on H^+ concentration gradients may be overly simplistic; relative buffer capacities and the balancing movements of strong electrolytes are probably also important. This whole area clearly deserves further investigation.

The metabolic fate of lactate

The classical mammalian view of lactic acid disposal and glycogen resynthesis is described by the Cori cycle. Muscle lactate is released into the bloodstream and taken up by the liver. Here the oxidation of a small fraction of the lactate provides the necessary ATP to resynthesize the major portion into glucose, which, in turn, is transported back to muscle to recharge glycogen reserves. This scenario has now been seriously questioned in mammals, and considerable evidence for oxidation or glyconeogenesis *in situ*, or in other muscles, has been now been gathered. The whole area remains controversial (see Gaesser and Brooks, 1984; Peters-Futre *et al.* 1987). In fish, a substantial body of evidence has now accumulated suggesting that the Cori cycle does not play an important role in the disposal of the post-exercise lactate burden. Whatever the exact route of metabolism or its contribution to EPOC, most of the lactate and H_m^+ appears to stay in the white muscle for metabolism *in situ*.

In brief, this evidence consists of the following points. (i) The bulk of the lactate and H_m^+ load is found in the white muscle at all times after exercise (see above). (ii) The ability of teleost hepatocytes to perform gluconeogenesis from lactate *in vitro* is minimal (Walsh *et al.* 1985, 1988; Mommsen *et al.* 1988). For example, in the toadfish, the measured rate of gluconeogenesis by hepatocytes could account for only 2 % of post-exercise lactate clearance (Walsh, 1989). (iii) In the only *in vivo* study of Cori cycle activity in a fish after exercise, Weber *et al.* (1986) reported that less than 5 % of labelled lactate was converted to glucose in skipjack tuna. In resting eels, this conversion was 35 % (Cornish and Moon, 1985). (iv) The turnover rate of the blood lactate pool, measured by the use of labelled lactate in tuna (Weber *et al.* 1986), salmon and flounder (Milligan and McDonald, 1988), was elevated after exhaustive exercise, but was still far too low to explain the observed rates of clearance of muscle lactate. This last piece of evidence would tend to rule against not only the Cori cycle, but any mechanism of lactate disposal based primarily on release to the bloodstream. (v) The turnover rate of the blood

glucose pool after exhaustive exercise, measured by the use of labelled glucose, was far too low to account for the observed rate of muscle glycogen restoration in the plaice (Batty and Wardle, 1979). Very recently, A. Pagnotta and C. L. Milligan (unpublished results) found that incorporation of labelled blood glucose accounted for less than 1% of resynthesized muscle glycogen in both trout and flounder. (vi) Finally, the original study of Batty and Wardle (1979) also indicated that labelled lactate, injected into the muscle of exhausted plaice, was directly resynthesized into glycogen. Exact quantification was problematical; this critical observation should be replicated.

The logical conclusion to be drawn from this evidence is that the bulk of lactate and H_m^+ are metabolized within the white muscle, either by oxidation or by glyconeogenesis. The small amounts released into the bloodstream are probably oxidized by other tissues such as liver (Mommsen *et al.* 1988), heart (Milligan and Farrell, 1991), red muscle (Bilinski and Jonas, 1972), gill (Mommsen, 1984) and RBCs (Wood *et al.* 1990*b*). The ability of teleost white muscle to oxidize lactate has also been documented (Bilinski and Jonas, 1972), though proof that a full complement of gluconeogenic enzymes exists in this tissue is still lacking.

The relative proportions cleared by the oxidative and glyconeogenic pathways *in situ*, and the proportion of glycogen resynthesized from lactate, may vary with species and with physiological condition. For example, in trout, the amount of lactate accumulated per unit of glycogen depleted varies greatly, depending on factors such as feeding condition (Scarabello *et al.* 1991*a*,*b*) and training (Pearson *et al.* 1990). Clearly, the proportion of glycogen that can be resynthesized from lactate will be limited by the size of the available lactate pool. The situation is further complicated by the fact that glycolysis may continue to produce lactate for some time after the end of exercise, even while lactate disposal is proceeding (Dobson and Hochachka, 1987; Lackner *et al.* 1988; Scarabello *et al.* 1991*a*,*b*; A. Pagnotta and C. L. Milligan, unpublished results). Acid–base status may be an important regulator of the fate of lactate. In trout, Milligan and Wood (1986*b*) demonstrated that the first 4 h of post-exercise recovery was a period of intense intracellular acidosis, during which there was considerable lactate and H_m^+ clearance from white muscle (Fig. 4A), apparently by oxidation. Restoration of glycogen levels started only once muscle pHi had been partially corrected. Catecholamines may be another important control; adrenergic stimulation of gluconeogenesis has been demonstrated in teleost hepatocytes (Mommsen *et al.* 1988; Danulat and Mommsen, 1990) and reptilian skeletal muscle (Gleeson and Kolok, 1990).

The metabolic effects of catecholamines after exercise

The preceding discussion has repeatedly implicated the post-exercise catecholamine surge as a modulator of responses during recovery. In mammals, general stimulatory effects of catecholamines on metabolism are thought to be another significant contributor to EPOC (Barnard and Foss, 1969; Gaesser and Brooks,

1984; Bahr *et al.* 1987). These actions may be either direct or indirect; that is, *v*[illegible] increased Na^+ and K^+ permeabilities (Horwitz, 1979) or by increased 'futile' substrate cycling (Newsholme, 1978). This raises the question of whether metabolic effects of catecholamines are a significant contributor to EPOC in fish.

In addressing this question, it must be remembered that the catecholamine surge occurs at a time of intense acidosis and internal disturbance, not under resting conditions. Both *in vivo* and *in vitro*, many earlier studies have shown that high levels of exogenous catecholamines will stimulate various aspects of aerobic metabolism (Mazeaud and Mazeaud, 1981). However, only recently has there been an emphasis on duplicating the *in vivo* conditions in terms of realistic post-exercise catecholamine levels, substrate levels and acid–base status. In general, these studies have demonstrated that altered acid–base status (decreased pHe and $[HCO_3^-]_e$, increased P_{CO_2}) and substrate levels (elevated blood glucose and lactate concentrations) are at least as important as catecholamines, if not more so, in accounting for observed changes in post-exercise metabolism.

For example, trout RBCs substantially increase their rate of lactate oxidation (and decrease their rate of glucose oxidation) during recovery from exhaustive exercise stress (Fig. 5A; Wood *et al.* 1990*b*; Walsh *et al.* 1990). *In vitro* factorial experiments demonstrated that more than half of this response was due to elevated blood [lactate], with additional smaller stimulations by elevated P_{CO_2} and elevated catecholamine levels. Interestingly, catecholamines exerted this stimulatory effect only in the presence of acidosis, and were without effect at normal blood pH. Total RBC $\dot{M}_{O_2}$ stayed more or less constant during recovery (Fig. 5B). However, $\dot{M}_{O_2}$ would have been markedly depressed by post-exercise acidosis, were it not for the presence of the elevated catecholamine levels, which restored it to normal levels. Note that RBC $\dot{M}_{O_2}$ was significantly depressed only at 2 h post-exercise, when the catecholamine surge had virtually disappeared, but acidosis persisted (Fig. 5B). Again, catecholamines had no effect at normal blood pH. Studies with other *in vitro* systems, such as the trout heart (Farrell and Milligan, 1986; Farrell *et al.* 1988), trout hepatocytes (Mommsen *et al.* 1988; Walsh *et al.* 1988; Perry *et al.* 1988) and toadfish hepatocytes (Walsh, 1989), have identified a variety of stimulatory or inhibitory effects of acid–base variables or substrate levels. In general, catecholamines appear to have little influence under control circumstances, when they would not be mobilized anyway. However, during post-exercise acidosis, they play an extremely important adaptive role in stimulating, protecting or modifying metabolism, but not in elevating tissue $\dot{M}_{O_2}$ above resting level.

In vivo, there have been no tests of the effects on whole-body $\dot{M}_{O_2}$ of catecholamine infusion or adrenergic blockade during post-exercise recovery. However, Butler *et al.* (1989) reported that infusion of physiological doses of adrenaline and noradrenaline slightly improved the aerobic swimming performance of cod in which endogenous catecholamine mobilization had been largely blocked by denervation of the head kidney. In resting trout, administration of exogenous adrenaline and noradrenaline, at doses either duplicating or exceedin[illegible]

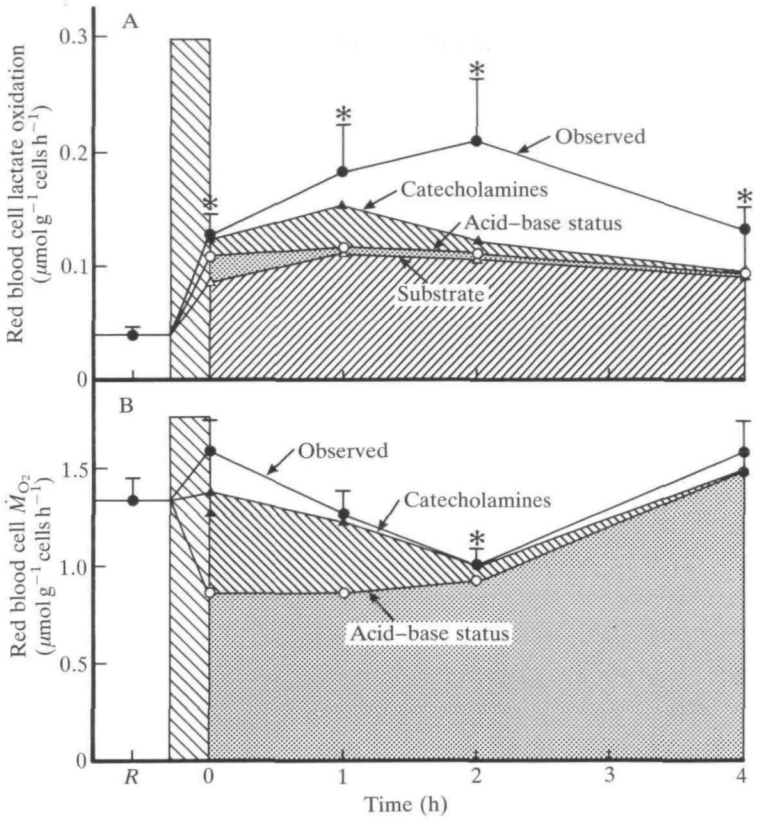

Fig. 5. A factorial analysis of the causes of observed *in vivo* changes in (A) the rate of production of CO_2 from lactate by red blood cells and (B) the rate of O_2 consumption ($\dot{M}_{O_2}$) of red blood cells during the first 4 h of recovery from exhaustive exercise stress (6 min chasing) in adult freshwater rainbow trout at 10°C. In A, the predicted rates as a result of measured changes in substrate concentration (lactate) alone, plus measured changes in acid–base status, plus measured changes in catecholamines are shown, based on the results of factorial modelling experiments *in vitro*. In B, a similar analysis is shown; substrate effects were not evaluated, though changes in [lactate] had no effect. Composite data from Walsh *et al.* (1990) and Wood *et al.* (1990*b*). Means +1 S.E.M., *N* numbers as in original papers. *R* indicates pre-exercise resting level; * indicates a mean value significantly different ($P<0.05$) from rest.

typical post-exercise levels, caused a negligible elevation of whole-body $\dot{M}_{O_2}$ (Steffensen *et al.* 1987; Playle *et al.* 1990). While there is clearly a need for more work both *in vivo* and *in vitro*, a tentative conclusion is that catecholamine effects on $\dot{M}_{O_2}$ are not an important contributor to EPOC in fish.

Post-exercise hyperventilation

Whatever factors are responsible for EPOC, it is essential that the fish maintain an increased ventilation ($\dot{V}$w) for some time during post-exercise recovery to

facilitate this elevated $\dot{M}_{O_2}$. The control of ventilation in fish has recently been reviewed elsewhere (Perry and Wood, 1989), so only points relevant to the post-exercise situation will be recapped here. The primary ventilatory drive is set by O_2, probably through detection of arterial blood O_2 content (Smith and Jones, 1982). However, during recovery from exhaustive exercise stress, arterial O_2 content is close to normal (Primmett *et al.* 1986; Milligan and Wood, 1987*a*) and the fish are generally motionless, so neither hypoxaemia nor proprioceptive input can be important stimuli for increased $\dot{V}w$. Catecholamines have been implicated in direct ventilatory stimulation (Peyraud- Waitzenegger 1979; Aota *et al.* 1990). However, recent studies in which physiological doses of adrenaline or noradrenaline were infused into resting or acidotic fish have shown negligible or hypoventilatory effects (Playle *et al.* 1990; Kinkead and Perry, 1990, 1991). In two elasmobranchs, the dogfish and the skate, blood acid–base status appears to play an important role in ventilatory control, probably acting through arterial pHa (Heisler, 1989; Wood *et al.* 1990*a*). If the same is true in all fish, then the large post-exercise acidosis of both metabolic and respiratory origin (Fig. 1A,B,C) may be the primary stimulus driving post-exercise hyperventilation, and catecholamines may play an important indirect role.

Both the cause and the functional significance of the respiratory component of post-exercise acidosis have been controversial (Wood and Perry, 1985; Perry, 1986; Perry and Vermette, 1987; Steffensen *et al.* 1987; Tufts *et al.* 1988; Playle *et al.* 1990). However, recently, Wood and Perry (1991) and Perry *et al.* (1991*b*) have confirmed that catecholamines inhibit RBC CO_2 excretion in trout *in vitro* by a β-adrenergic mechanism linked to Na^+/H^+ exchange on the erythrocyte membrane. The same inhibition is seen *in vivo* during post-exercise recovery (C. M. Wood and R. S. Munger, unpublished results). In effect, the mechanism that protects RBC pHi and blood O_2 transport (Nikinmaa, 1982; Nikinma *et al.* 1984, 1990; Primmett *et al.* 1986) simultaneously reduces the rate of HCO_3^- dehydration by RBCs passing through the gills, causing CO_2 to back up in the animal. A marked increase in Pa_{CO_2} and decrease in pHa result (Fig. 1A,C). The phenomenon appears to be coupled to the decrease or reversal of the extracellular–intracellular pH gradient across the RBC membrane (Wood and Perry, 1991). All fish probably exhibit a decrease in this pH gradient after exhaustive exercise stress, simply as a result of the different buffer capacities of the two compartments. However, it remains to be seen whether this effect alone is sufficient to explain the post-exercise increase in Pa_{CO_2} exhibited by species where the adrenergically regulated Na^+/H^+ is weak or lacking (e.g. elasmobranchs; Holeton and Heisler, 1983).

In any event, the respiratory component exacerbates the metabolic acidosis (due to H_m^+ movement out of muscle and RBCs) in all fish that have been examined (Wood and Perry, 1985). This large compound acidosis probably serves to stimulate $\dot{V}w$ after exercise in the absence of other appropriate stimuli. Recent experiments on trout support this interpretation (C. M. Wood and R. S. Munger, unpublished results). Relative $\dot{V}w$ during recovery from exhaustive stress i

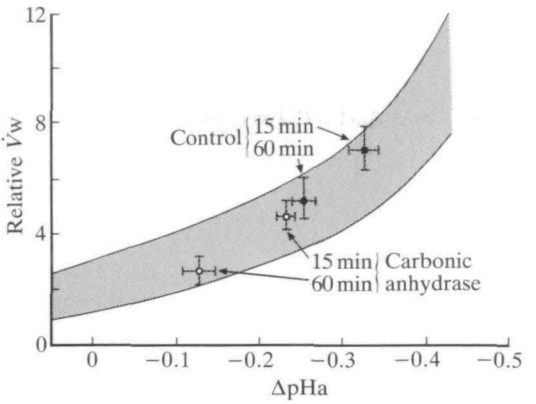

Fig. 6. The mean relationship (±1 S.E.M.) between relative ventilation ($\dot{V}$w) and the change in arterial blood pH (ΔpHa) determined from 91 measurements in 27 adult rainbow trout at various times after exhaustive exercise stress (6 min chasing) at 15°C. $\dot{V}$w and ΔpHa are expressed relative to pre-exercise resting levels. Superimposed on this relationship are shown the data (means±1 S.E.M.) for the two different treatment groups at 15 and 60 min post-exercise. The experimental group (N=12) received an intra-arterial infusion of 10 mg kg^{-1} bovine carbonic anhydrase to reduce post-exercise increases in Pa_{CO_2} and decreases in pHa. The control group (N=15) received only saline. (Unpublished data of C. M. Wood and R. S. Munger.)

related to the decline in pHa in a simple exponential fashion (Fig. 6). Intra-arterial injection of sufficient bovine carbonic anhydrase to double the net HCO_3^--dehydration activity of the blood does not affect exercise performance, but reduces the post-exercise elevation in Pa_{CO_2} by about 50%. As a result, the decline in pHa is greatly reduced; this, in turn, is correlated with a substantial reduction in the extent of post-exercise hyperventilation (Fig. 6) and therefore in $\dot{M}_{O_2}$. In this context, post-exercise acidosis appears to be adaptive in sustaining elevated $\dot{V}$w to meet the demands of EPOC.

Conclusion

In an earlier review (Wood and Perry, 1985), we pointed to the need for direct determinations of plasma catecholamine levels to support the suspected involvement of these stress hormones in a variety of responses to exercise. The intervening 6 years have seen a substantial increase in such measurements, as well as in tests of realistic doses of catecholamines under realistic conditions. From the preceding discussion, it is now clear that circulating catecholamines are critically involved in many facets of post-exercise physiology. Overall, elevations in circulating adrenaline and noradrenaline levels are probably more important in recovery from exhaustive exercise stress than they are in steady-state aerobic swimming. The next stage is to probe the cellular bases of these effects, and then to integrate them back into the response of the whole animal. In this regard, recent

reports that adrenoreceptor density can be rapidly adjusted in stress situations are particularly intriguing (Marttila and Nikinmaa, 1988; Reid and Perry, 1991).

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References

AOTA, S., HOLMGREN, K. D., GALLAUGHER, P. AND RANDALL, D. J. (1990). A possible role for catecholamines in the ventilatory responses associated with internal acidosis or external hypoxia in rainbow trout *Oncorhynchus mykiss*. *J. exp. Biol.* **151**, 57–70.

AXELSSON, M. AND NILSSON, S. (1986). Blood pressure control during exercise in the Atlantic cod *Gadus morhua*. *J. exp. Biol.* **126**, 225–236.

BAHR, R., INGNES, I., VAAGE, O., SEJERSTED, O. M. AND NEWSHOLME, E. (1987). Effect of duration of exercise on excess postexercise O_2 consumption. *J. appl. Physiol.* **62**, 485–490.

BARNARD, R. J. AND FOSS, M. L. (1969). Oxygen debt: effect of beta-adrenergic blockade on the lactacid and alactacid components. *J. appl. Physiol.* **27**, 813–816.

BARTON, B. A., SHRECK, C. B. AND SIGISMONDI, L. A. (1986). Multiple acute disturbances evoke cumulative physiological stress responses in juvenile chinook salmon. *Trans. Am. Fish. Soc.* **115**, 245–251.

BATTY, R. S. AND WARDLE, C. S. (1979). Restoration of glycogen from lactic acid in the anaerobic swimming muscle of plaice, *Pleuronectes platessa* L. *J. Fish Biol.* **15**, 509–519.

BEGGS, G. L., HOLETON, G. F. AND CROSSMAN, E. J. (1980). Some physiological consequences of angling stress in muskellunge, *Esox masquinongy* Mitchill. *J. Fish Biol.* **17**, 649–660.

BILINSKI, E. AND JONAS, R. E. E. (1972). Oxidation of lactate to carbon dioxide by rainbow trout (*Salmo gairdneri*) tissues. *J. Fish. Res. Bd Can.* **29**, 1467–1471.

BLACK, E. C. (1958). Hyperactivity as a lethal factor in fish. *J. Fish. Res. Bd Can.* **15**, 245–251.

BLACK, E. C., ROBERTSON, A. C., LAM, K.-C. AND CHIU, W.-G. (1962). Changes in glycogen, pyruvate and lactate in rainbow trout (*Salmo gairdneri*) during and following muscular activity. *J. Fish. Res. Bd Can.* **19**, 409–436.

BOUCK, G. R. AND BALL, R. C. (1966). Influence of capture methods on blood characteristsics and mortality in the rainbow trout (*Salmo gairdneri*). *Trans. Am. Fish. Soc.* **95**, 170–176.

BOUTILIER, R. G., AUGHTON, P. AND SHELTON, G. (1984). O_2 and CO_2 transport in relation to ventilation in the Atlantic mackerel, *Scomber scombrus*. *Can. J. Zool.* **62**, 546–554.

BOUTILIER, R. G., IWAMA, G. K. AND RANDALL, D. J. (1986). The promotion of catecholamine release in rainbow trout, *Salmo gairdneri*, by acute acidosis: interactions between red cell pH and haemoglobin oxygen-carrying capacity. *J. exp. Biol.* **123**, 145–157.

BRETT, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Bd Can.* **21**, 1183–1226.

BUTLER, P. J., AXELSSON, M., EHRENSTROM, F., METCALFE, J. D. AND NILSSON, S. (1989). Circulating catecholamines and swimming performance in the Atlantic cod, *Gadus morhua*. *J. exp. Biol.* **141**, 377–387.

BUTLER, P. J., METCALFE, J. D. AND GINLEY, S. A. (1986). Plasma catecholamines in the lesser spotted dogfish and rainbow trout at rest and during different levels of exercise. *J. exp. Biol.* **123**, 409–421.

CORNISH, I. AND MOON, T. W. (1985). Glucose and lactate kinetics in American eel, *Anguilla rostrata*. *Am. J. Physiol.* **249**, R67–R72.

DANULAT, E. AND MOMMSEN, T. P. (1990). Norepinephrine: a potent activator of glycogenolysis and gluconeogenesis in rockfish hepatocytes. *Gen. comp. Endocr.* **78**, 12–22.

DOBSON, G. P. AND HOCHACHKA, P. W. (1987). Role of glycolysis in adenylate depletion and repletion during work and recovery in teleost white muscle. *J. exp. Biol.* **129**, 125–140.

DOBSON, G. P., PARKHOUSE, W. S. AND HOCHACHKA, P. W. (1987). Regulation of anaerobic ATP-generating pathways in trout fast-twitch skeletal muscle. *Am. J. Physiol.* **253** R186–R194.

DRIEDZIC, W. R. AND HOCHACHKA, P. W. (1976). Control of energy metabolism in fish white muscle. *Am. J. Physiol.* **230**, 579–582.

DRIEDZIC, W. R. AND KICENIUK, J. W. (1976). Blood lactate levels in free-swimming rainbow trout (*Salmo gairdneri*) before and after strenuous exercise resulting in fatigue. *J. Fish. Res. Bd Can.* **33**, 173–176.

DUTHIE, G. G. (1982). The respiratory metabolism of temperature-adapted flatfish at rest and during swimming activity and the use of anaerobic metabolism at moderate swimming speeds. *J. exp. Biol.* **97**, 359–373.

FARMER, G. J. AND BEAMISH, F. W. H. (1969). Oxygen consumption of *Tilapia nilotica* in relation to swimming speed and salinity. *J. Fish. Res. Bd Can.* **26**, 2807–2821.

FARRELL, A. P., MACLEOD, K. R. AND SCOTT, C. (1988). Cardiac performance of the trout (*Salmo gairdneri*) heart during acidosis; effects of low bicarbonate, lactate and cortisol. *Comp. Biochem. Physiol.* **91**A, 271–277.

FARRELL, A. P. AND MILLIGAN, C. L. (1986). Myocardial intracellular pH in a perfused rainbow trout heart during extracellular acidosis in the presence and absence of adrenalin. *J. exp. Biol.* **125**, 347–359.

FERGUSON, R.A. AND BOUTILIER, R.G. (1989). Metabolic–membrane coupling in red blood cells of trout: the effects of anoxia and adrenergic stimulation. *J. exp. Biol.* **143**, 149–164.

FIEVET, B., CAROFF, J. AND MOTAIS, R. (1990). Catecholamine release by blood oxygen tension during deep hypoxia in trout: effect on red blood cell Na^+/H^+ exchanger activity. *Respir. Physiol.* **79**, 81–90.

FRASER, J. M. AND BEAMISH, F. W. H. (1969). Blood lactic acid concentrations in brook trout, *Salvelinus fontinalis*, planted by air drop. *Trans. Am. Fish. Soc.* **2**, 263–267.

GAESSER, G. A. AND BROOKS, G. A. (1984). Metabolic bases of excess post-exercise oxygen consumption: a review. *Med. Sci. Sports Exercise* **16**, 29–43.

GLEESON, T. AND KOLOK, A. S. (1990). Skeletal muscle gluconeogenesis: glycogen synthesis from lactate is stimulated by epinephrine. *Physiologist* **33**, A36.

GOSS, G. G. AND WOOD, C. M. (1991). Two-substrate kinetic analysis: a novel approach linking ion and acid–base transport at the gills of freshwater trout. *J. comp. Physiol.* B (in press).

HEISLER, N. (1989). Acid–base regulation. In *Physiology of Elasmobranch Fishes* (ed. T. J. Shuttleworth), pp. 215–252. Berlin: Springer-Verlag.

HILL, A. V. AND LUPTON, H. (1923). Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Q. J. Med.* **16**, 135–171.

HOCHACHKA, P. W. AND MOMMSEN, T. P. (1983). Protons and anaerobiosis. *Science* **219**, 1391–1397.

HOFMANN, E. L. AND BUTLER, D. G. (1979). The effect of increased metabolic rate on renal function in the rainbow trout, *Salmo gairdneri*. *J. exp. Biol.* **82**, 11–23.

HOLETON, G.F. AND HEISLER, N. (1983). Contribution of net ion transfer mechanisms to acid–base regulation after exhausting activity in the larger spotted dogfish (*Scyliorhinus stellaris*). *J. exp. Biol.* **103**, 31–46.

HOLETON, G. F., NEUMANN, P. AND HEISLER, N. (1983). Branchial ion exchange and acid–base regulation after strenuous exercise in rainbow trout (*Salmo gairdneri*). *Respir. Physiol.* **51**, 303–318.

HORWITZ, B. A. (1979). Metabolic aspects of thermogenesis; neuronal and hormonal control. *Fedn Proc. Fedn Am. Socs exp. Biol.* **38**, 2147–2149.

HUGHES, G. M., LE BRAS-PENNEC, T. AND PENNEC, J. P. (1988). Relationship between swimming speed, oxygen consumption, plasma catecholamines, and heart performance in rainbow trout (*S. gairdneri* R.) *Exp. Biol.* **48**, 45–49.

JENSEN, F. B., NIKINMAA, M. AND WEBER, R. E. (1983). Effects of exercise stress on acid–base balance and respiratory function in blood of the teleost *Tinca tinca*. *Respir. Physiol.* **51**, 291–301.

JOHNSTON, I. A. AND GOLDSPINK, G. (1973). A study of glycogen and lactate in the myotomal muscles and liver of the coalfish (*Gadus virens* L.) during sustained swimming. *J. mar. Biol. Ass. U.K.* **53**, 17–26.

KICENIUK, J. W. AND JONES, D. R. (1977). The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *J. exp. Biol.* **69**, 247–260.

KINKEAD, R. AND PERRY, S. F. (1990). An investigation of the role of circulating catecholamines

in the control of ventilation during acute moderate hypoxia in rainbow trout (*Oncorhynchus mykiss*). *J. comp. Physiol.* B **160**, 441–448.

KINKEAD, R. AND PERRY, S. F. (1991). The effects of intra-arterial infusion of catecholamines on ventilation in rainbow trout (*Oncorhynchus mykiss*) exposed to external hypoxia or hypercapnia. *Respir. Physiol.* (in press).

KOWALCHUK, J. M., HEIGENHAUSER, G. F., LINDINGER, M. I., SUTTON, J. R. AND JONES, N. L. (1988). Factors influencing hydrogen ion concentration in muscle after intense exercise. *J appl. Physiol.* **65**, 2080–2089.

LACKNER, R., WIESER, W., HUBER, M. AND DALLA VIA, J. (1988). Responses of intermediary metabolism to acute handling stress and recovery in untrained and trained *Leuciscus cephalus* (Cyprinidae, Teleostei). *J. exp. Biol.* **140**, 393–404.

MARGARIA, R., EDWARDS, H. T. AND DILL, D. B. (1933). The possible mechanisms of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. *Am. J..Physiol.* **106**, 689–715.

MARTTILA, O. N. T. AND NIKINMAA, M. (1988). Binding of β-adrenergic antagonists ^{3}H-DHA and ^{3}H-GGP 12177 to intact rainbow trout (*Salmo gairdneri*) and carp (*Cyprinus carpio*) red blood cells. *Gen. comp. Endocr.* **70**, 429–435.

MAZEAUD, M. M. AND MAZEAUD, F. (1981). Adrenergic responses to stress in fish. In *Stress and Fish* (ed. A. D. Pickering), pp. 49–75. London: Academic Press.

MCDONALD, D. G., TANG, Y. AND BOUTILIER, R. G. (1989*a*). The role of β-adrenoreceptors in the recovery from exhaustive exercise in freshwater-adapted rainbow trout. *J. exp. Biol.* **147**, 471–491.

MCDONALD, D. G., TANG, Y. AND BOUTILIER, R. G. (1989*b*). Regulation of acid and ion transfer across the gills of fish. *Can. J. Zool.* **67**, 3046–3054.

MESA, M. G. AND SHRECK, C. B. (1989). Electro-fishing mark recapture and depletion methodologies evoke behavioural and physiological changes in cutthroat trout. *Trans. Am. Fish. Soc.* **118**, 644–658.

MILLIGAN, C. L. AND FARRELL, A. P. (1986). Extracellular and intracellular acid–base status following strenuous activity in the sea raven (*Hemitripterus americanus*). *J. comp. Physiol.* **156**B, 583–590.

MILLIGAN, C. L. AND FARRELL, A. P. (1991). Lactate utilization by an *in situ* perfused trout heart: effects of workload and blockers of lactate transport. *J. exp. Biol.* **155**, 357–373.

MILLIGAN, C. L., GRAHAM, M. S. AND FARRELL, A. P. (1989). The response of trout red cells to adrenaline during seasonal acclimation and changes in temperature. *J. exp. Biol.* **35**, 229–236.

MILLIGAN, C. L. AND MCDONALD, D. G. (1988). *In vivo* lactate kinetics at rest and during recovery from exhaustive exercise in coho salmon (*Oncorhynchus kisutch*) and starry flounder (*Platichthys stellatus*). *J. exp. Biol.* **135**, 199–131.

MILLIGAN, C. L., MCDONALD, D. G. AND PRIOR, E. T. (1991). Branchial acid and ammonia fluxes in response to alkalosis and acidosis in two marine teleosts: coho salmon (*Oncorhynchus kisutch*) and starry flounder (*Platichthys stellatus*). *Physiol. Zool.* **64**, 169–192.

MILLIGAN, C. L. AND WOOD, C. M. (1986*a*). Intracellular and extracellular acid–base status and H^+ exchange with the environment after exhaustive exercise in the rainbow trout. *J. exp. Biol.* **123**, 93–121.

MILLIGAN, C. L. AND WOOD, C. M. (1986*b*). Tissue intracellular acid–base status and the fate of lactate after exhaustive exercise in the rainbow trout. *J. exp. Biol.* **123**, 123–144.

MILLIGAN, C. L. AND WOOD, C. M. (1987*a*). Regulation of blood oxygen transport and red cell pHi after exhaustive activity in rainbow trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). *J. exp. Biol.* **133**, 263–282.

MILLIGAN, C. L. AND WOOD, C. M. (1987*b*). Effects of strenuous activity in intracellular and extracellular acid–base status and H^+ exchange with the environment in the inactive, benthic starry flounder, *Platichthys stellatus*. *Physiol. Zool.* **60**, 37–53.

MILLIGAN, C. L. AND WOOD, C. M. (1987*c*). Muscle and liver intracellular acid–base and metabolite status after strenuous activity in the inactive, benthic starry flounder (*Platichthys stellatus*). *Physiol. Zool.* **60**, 54–68.

MOMMSEN, T. P. (1984). Biochemical characterization of the rainbow trout gill. *J. comp Physiol.* B **154**, 191–198.

MOMMSEN, P. AND HOCHACHKA, P. W. (1988). The purine nucleotide cycle as two temporarily separated metabolics units: a study on trout muscle. *Metabolism* **37**, 552–556.

MOMMSEN, T. P., WALSH, P. J., PERRY, S. F. AND MOON, T. W. (1988). Interactive effects of catecholamines and hypercapnia on glucose production in isolated trout hepatocytes. *Gen. comp. Endrocr.* **70**, 63–73.

NEILSEN, J. D., WAIWOOD, K. G. AND SMITH, S. J. (1989). Survival of Atlantic halibut (*Hippoglossus hippoglossus*) caught by long-line and otter trawl gear. *Can. J. Fish. aquat. Sci.* **46**, 887–897.

NEUMANN, P., HOLETON, G. F. AND HEISLER, N. (1983). Cardiac output and regional blood flow in gills and muscles after exhaustive exercise in rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **105**, 1–14.

NEWSHOLME, E. A. (1978). Substrate cycles: their metabolic, energetic, and thermic consequences in man. *Biochem. Soc. Symp.* **43**, 183–205.

NIKINMAA, M. (1982). Effects of adrenaline on red cell volume and concentration gradient of protons across the red cell membrane in the rainbow trout, *Salmo gairdneri*. *Molec. Physiol.* **2**, 287–297.

NIKINMAA, M., CECH, J. J., JR AND MCENROE, M. (1984). Blood oxygen transport in stressed striped bass (*Morone saxatilis*): role of beta-adrenergic responses. *J. comp. Physiol.* B **154**, 365–369.

NIKINMAA, M., TIIHONEN, K. AND PAAJASTE, M. (1990). Adrenergic control of red cell pH in salmonid fish: roles of the sodium/proton exchange, Jacobs–Stewart cycle and membrane potential. *J. exp. Biol.* **154**, 257–271.

NILSSON, S., ABRAHAMSSON, T. AND GROVE, D. J. (1976). Sympathetic nervous control of adrenaline release from the head kidney of the cod *Gadus morhua*. *Comp. Biochem. Physiol.* **55**C, 123–127.

PARKER, R. R. AND BLACK, E. C. (1959). Muscular fatigue and mortality in troll caught chinook salmon (*Oncorhynchus tshawytascha*). *J. Fish. Res. Bd Can.* **16**, 95–106.

PARKHOUSE, W. S., DOBSON, G. P., BELCASTRO, A. N. AND HOCHACHKA, P. W. (1987). The role of intermediary metabolism in the maintenance of proton and charge balance during exercise. *Molec. cell. Biochem.* **77**, 37–47.

PARKHOUSE, W. S., DOBSON, G. P. AND HOCHACHKA, P. W. (1988). Control of glycogenolysis in rainbow trout muscle during exercise. *Can. J. Zool.* **66**, 345–351.

PEARSON, M. P., SPRIET, L. L. AND STEVENS, E. D. (1990). Effect of sprint training on swim performance and white muscle metabolism during exercise and recovery in rainbow trout. *J. exp. Biol.* **149**, 45–60.

PERRY, S. F. (1986). Carbon dioxide excretion in fishes. *Can. J. Zool.* **64**, 565–572.

PERRY, S. F., DAXBOECK, C., EMMETT, B., HOCHACHKA, P. W. AND BRILL, R. W. (1985). Effects of exhausting exercise on acid–base regulation in skipjack (*Katsuwonus pelomis*) blood. *Physiol. Zool.* **58**, 421–429.

PERRY, S. F., FRITSCHE, R., KINKEAD, R. AND NILSSON, S. (1991*a*). Control of catecholamine release *in vivo* and *in situ* in the Atlantic cod (*Gadus morhua*) during hypoxia. *J. exp. Biol.* **155**, 549–566.

PERRY, S. F., KINKEAD, R., GALLAUGHER, P. AND RANDALL, D. J. (1989). Evidence that hypoxemia promotes catecholamine release during hypercapnic acidosis in rainbow trout (*Salmo gairdneri*). *Respir. Physiol.* **77**, 351–364.

PERRY, S. F. AND VERMETTE, M. G. (1987). The effects of prolonged epinephrine infusion on the physiology of the rainbow trout, *Salmo gairdneri*. I. Blood respiratory, acid–base and ionic status. *J. exp. Biol.* **128**, 235–253.

PERRY, S. F., WALSH, P. J., MOMMSEN, T. P. AND MOON, T. W. (1988). Metabolic consequences of hypercapnia in the rainbow trout, *Salmo gairdneri*: β-adrenergic effects. *Gen. comp. Endocr.* **69**, 439–447.

PERRY, S. F. AND WOOD, C. M. (1989). Control and coordination of gas transfer in fishes. *Can. J. Zool.* **67**, 2961–2970.

PERRY, S. F., WOOD, C. M., THOMAS, S. AND WALSH, P. J. (1991*b*). Adrenergic inhibition of carbon dioxide excretion by trout red blood cells *in vitro* is mediated by activation of Na^+/H^+ exchange. *J. exp. Biol.* **157**, 367–380

PETERS-FUTRE, E. M., NOAKES, T. D., RAINE, R. I. AND TERBLANCHE, S. E. (1987). Muscle glycogen repletion during active post-exercise recovery. *Am. J. Physiol.* **253**, E305–E311.

PEYRAUD-WAITZENEGGER, M. (1979). Simultaneous modifications of ventilation and arterial P_{O_2} by catecholamines in the eel, *Anguilla anguilla L.*: participation of α and β effects. *J. comp. Physiol.* **129**, 343–354.

PIIPER, J., MEYER, M. AND DREES, F. (1972). Hydrogen ion balance in the elasmobranch *Scyliorhinus stellaris* after exhausting activity. *Respir. Physiol.* **16**, 290–303.

PLAYLE, R. C., MUNGER, R. S. AND WOOD, C. M. (1990). Effects of catecholamines on gas exchange and ventilation in rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **152**, 353–367.

PRIMMETT, D. R. N., RANDALL, D. J., MAZEAUD, M. AND BOUTILIER, R. G. (1986). The role of catecholamines in erythrocyte pH regulation and oxygen transport in rainbow trout (*Salmo gairdneri*) during exercise. *J. exp. Biol.* **122**, 139–148.

RANDALL, D. J. AND BRAUNER, C. (1991). Effects of environmental factors on exercise in fish. *J. exp. Biol.* **160**, 113–126.

RAO, G. M. M. (1969). Effect of activity, salinity and temperature on plasma concentrations of rainbow trout. *Can. J. Zool.* **47**, 131–134.

REID, S. D. AND PERRY, S. F. (1991). The effects and physiological consequences of raised levels of cortisol on rainbow trout (*Oncorhynchus mykiss*) erythrocyte β-adrenoreceptors. *J. exp. Biol.* (in press).

RISTORI, M. T. AND LAURENT, P. (1985). Plasma catecholamines and glucose during moderate exercise in the trout: comparison with bursts of violent activity. *Exp. Biol.* **44**, 247–253.

SCARABELLO, M., HEIGENHAUSER, G. H. J. AND WOOD, C. M. (1991*a*). The oxygen debt hypothesis in juvenile rainbow trout after exhaustive exercise. *Respir. Physiol.* (in press).

SCARABELLO, M., WOOD, C. M. AND HEIGENHAUSER, G. H. J. (1991*b*). Glycogen depletion as an experimental test of the oxygen debt hypothesis in juvenile rainbow trout. *Can. J. Zool.* (in press).

SCHRECK, C. B., WHALEY, R. A., BASS, M. L., MAUGHAN, O. E. AND SOLAZZI, M. (1976). Physiological responses of rainbow trout (*Salmo gairdneri*) to electroshock. *J. Fish. Res. Bd Can.* **33**, 76–84.

SCHWALME, K. AND MACKAY, W. C. (1985*a*). The influence of exercise-handling stress on blood lactate, acid–base, and plasma glucose status of northern pike (*Esox lucius* L.) *Can. J. Zool.* **63**, 1125–1129.

SCHWALME, K. AND MACKAY, W. C. (1985*b*). The influence of angling-induced exercise on the carbohydrate metabolism of the northern pike (*Esox lucius* L.). *J. comp. Physiol.* B **156**, 67–75.

SJOGAARD, G. (1986). Water and electrolyte fluxes during exercise and their relation to muscle fatigue. *Acta physiol. scand.* **128** (Suppl. 556), 129–136.

SMITH, F. M. AND JONES, D. R. (1982). The effect of changes in blood oxygen carrying capacity on ventilation volume in the rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **97**, 325–334.

STEFFENSEN, J. F., TUFTS, B. L. AND RANDALL, D. J. (1987). Effect of burst swimming and adrenaline infusion on O_2 consumption and CO_2 excretion in rainbow trout *Salmo gairdneri*. *J. exp. Biol.* **131**, 427–434.

TANG, Y. AND BOUTILIER, R. G. (1988). Correlation between catecholamine release and degree of acidotic stress in trout. *Am. J. Physiol.* **255**, R395–R399.

TANG, Y., MCDONALD, D. G. AND BOUTILIER, R. G. (1989). Adrenergic regulation of blood acid–base status following exhaustive exercise in seawater-adapted rainbow trout, *Salmo gairdneri*. *Physiol. Zool.* **62**, 950–963.

THOMAS, S., POUPIN, J., LYKKEBOE, G. AND JOHANSEN, K. (1987). Effects of graded exercise on blood gas tensions and acid–base characteristics of rainbow trout. *Respir. Physiol.* **68**, 85–97.

TUFTS, B. L., FERGUSON, R. A. AND BOUTILIER, R. G. (1988). *In vivo* and *in vitro* effects of adrenergic stimulation on chloride/bicarbonate exchange in rainbow trout erythrocytes. *J. exp. Biol.* **140**, 301–312.

TURNER, J. D. AND WOOD, C. M. (1983). Factors affecting lactate and proton efflux from pre-exercised, isolated-perfused rainbow trout trunks. *J. exp. Biol.* **105**, 395–401.

TURNER, J. D., WOOD, C. M. AND CLARK, D. (1983*a*). Lactate and proton dynamics in the rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **104**, 247–268.

TURNER, J. D., WOOD, C. M. AND HÖBE, H. (1983*b*). Physiological consequences of severe

exercise in the inactive benthic flathead sole (*Hippoglossoides elassodon*): a comparison with the active pelagic rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **104**, 269–288.

VAN DIJK, P. AND WOOD, C. M. (1988). The effect of β-adrenergic blockade on the recovery process after strenuous exercise in the rainbow trout (*Salmo gairdneri* Richardson). *J. Fish Biol.* **32**, 557–570.

VERMETTE, M. G. AND PERRY, S. F. (1987). The effects of prolonged epinephrine infusion on the physiology of the rainbow trout, *Salmo gairdneri*. II. Branchial solute fluxes. *J. exp. Biol.* **128**, 255–267.

WALSH, P. J. (1989). An *in vitro* model of post-exercise hepatic gluconeogenesis in the gulf toadfish *Opsanus beta*. *J. exp. Biol.* **147**, 393–406.

WALSH, P. J., MOMMSEN, T. P., MOON, T. W. AND PERRY, S. F. (1988). Effects of acid–base variables on *in vitro* hepatic metabolism in rainbow trout. *J. exp. Biol.* **135**, 231–241.

WALSH, P. J., MOON, T. W. AND MOMMSEN, T. P. (1985). Interactive effects of acute changes in temperature and pH on metabolism in hepatocytes from the sea raven *Hemitripterus americanus*. *Physiol. Zool.* **58**, 727–735.

WALSH, P. J., WOOD, C. M., THOMAS, S. AND PERRY, S. F. (1990). Characterization of red blood cell metabolism in rainbow trout. *J. exp. Biol.* **154**, 475–489.

WARDLE, C. S. (1978). Non-release of lactic acid from anaerobic swimming muscle of plaice, *Pleuronectes platessa* L: a stress reaction. *J. exp. Biol.* **77**, 141–155.

WEBER, J. M., BRILL, R. W. AND HOCHACHKA, P. W. (1986). Mammalian metabolite flux rates in a teleost: lactate and glucose turnover in tuna. *Am. J. Physiol.* **250**, R452–R458.

WELLS, R. M. G., MCINTYRE, R. H., MORGAN, A. K. AND DAVIE, P. S. (1986). Physiological stress responses in big gamefish after capture: observations on plasma chemistry and blood factors. *Comp. Biochem. Physiol.* A **84**, 565–571.

WIESER, W., PLATZER, U. AND HINTERLEITNER, S. (1985). Anaerobic and aerobic energy production of young rainbow trout (*Salmo gairdneri*) during and after bursts of activity. *J. comp. Physiol.* B **155**, 485–492.

WOKOMA, A. AND JOHNSTON, I. A. (1981). Lactate production at high sustainable cruising speeds in rainbow trout (*Salmo gairdneri* Richardson). *J. exp. Biol.* **90**, 361–364.

WOOD, C. M. (1988). Acid–base and ionic exchanges at gills and kidney after exhaustive exercise in the rainbow trout. *J. exp. Biol.* **136**, 461–481.

WOOD, C. M., MCMAHON, B. R. AND MCDONALD, D. G. (1977). An analysis of changes in blood pH following exhausting activity in the starry flounder, *Platichthys stellatus*. *J. exp. Biol.* **69**, 173–185.

WOOD, C. M. AND MILLIGAN, C. L. (1987). Adrenergic analysis of extracellular and intracellular lactate and H^+ dynamics after strenuous exercise in the starry flounder, *Platichthys stellatus*. *Physiol. Zool.* **60**, 69–81.

WOOD, C.M. AND PERRY, S.F. (1985). Respiratory, circulatory, and metabolic adjustments to exercise in fish. In *Circulation, Respiration, Metabolism* (ed. R. Gilles), pp. 2–22. Berlin: Springer-Verlag.

WOOD, C. M. AND PERRY, S. F. (1991). A new *in vitro* assay for carbon dioxide excretion by trout red blood cells: effects of catecholamines. *J. exp. Biol.* **157**, 349–366.

WOOD, C. M. AND RANDALL, D. J. (1973*a*). The influence of swimming activity on sodium balance in the rainbow trout (*Salmo gairdneri*). *J. comp. Physiol.* **82**, 207–233.

WOOD, C. M. AND RANDALL, D. J. (1973*b*). Sodium balance in the rainbow trout (*Salmo gairdneri*) during extended exercise. *J. comp. Physiol.* **82**, 235–256.

WOOD, C. M. AND RANDALL, D. J. (1973*c*). The influence of swimming activity on water balance in the rainbow trout (*Salmo gairdneri*). *J. comp. Physiol.* **82**, 257–276.

WOOD, C. M., TURNER, J. D. AND GRAHAM, M. S. (1983). Why do fish die after severe exercise? *J. Fish. Biol.* **22**, 189–201.

WOOD, C. M., TURNER, J. D., MUNGER, R. S. AND GRAHAM, M. S. (1990*a*). Control of ventilation in the hypercapnic skate *Raja ocellata*. II. Cerebrospinal fluid and intracellular pH in the brain and other tissues. *Respir. Physiol.* **80**, 279–298.

WOOD, C. M., WALSH, P. J., THOMAS, S. AND PERRY, S. F. (1990*b*). Control of red blood cell metabolism in rainbow trout after exhaustive exercise. *J. exp. Biol.* **154**, 491–507.

WOODWARD, C. C. AND STRANGE, R. J. (1987). Physiological stress responses in wild and hatchery-reared rainbow trout. *Trans. Am. Fish. Soc.* **116**, 574–579.

WRIGHT, P. A., RANDALL, D. J. AND WOOD, C. M. (1988). The distribution of ammonia and H^+ between tissue compartments in lemon sole (*Parophrys vetulus*) at rest, during hypercapnia and following exercise. *J. exp. Biol.* **136**, 149–75.

WYDOSKI, R. S., WEDEMEYER, G. A. AND NELSON, N. C. (1976). Physiological response to hooking stress in hatchery and wild rainbow trout (*Salmo gairdneri*). *Trans. Am. Fish. Soc.* **105**, 601–606.